APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1978 p. 610-613
0099-2240/78/0035-0610$02.00/0

Vol. 35, No. 2
Printed in U.S.A.

NOTES

Isolation and Cultivation of Spirochetes and Other Spiral-Shaped Bacteria Associated with the Cecal Mucosa of Rats and Mice

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Received for publication 12 September 1977

A method for the culture of spiral-shaped bacteria associated with the intestinal mucosa of rodents is described. The appearance in culture of a spiral organism from rat ceca and a spirochete from mouse ceca is illustrated; these organisms are morphologically similar to the major inhabitants of the cecal mucosa in each animal species.

Studies on the microbial ecology of the gastrointestinal tract have demonstrated large populations of microorganisms in the mucous layer of epithelial surfaces lining the lumen of the intestine or deep down in the crypts of the large bowel (3, 7, 9, 10). Because of their close association with the gut surface, these bacteria presumably contribute to the resistance of the host to invasion by intestinal pathogens. The introduction of anaerobic role tube techniques or the use of anaerobic gloveboxes has resulted in the cultivation of fusiform bacteria that inhabit the mucous layer of the cecum and colon of rodents (6, 8). However, isolation and growth of a group of spiral-shaped organisms that are present in the crypts of many animal species have proved difficult (6). These bacteria are the organisms most intimately associated with host tissue, even to the extent that they can be seen within the goblet cells of the cecal mucosa.

In this note we describe the isolation and culture, for the first time, of spiral organisms from the cecal crypts of mice and rats.

Adult animals were killed by spinal dislocation. A small piece of the cecum of each animal was placed on a sheet of clean paper towelling, the mucosa was exposed, and the lumen contents were removed by lightly scraping the mucosal surface with the blunt edge of a pair of scissors. The piece of tissue was held firmly with forceps and vigorously agitated back and forth 30 times in 50 ml of physiological saline in a 100-ml beaker. This washing procedure was repeated nine times in fresh saline. The washed tissue was placed on the surface of a plastic petri dish with the epithelial surface uppermost. The mucosa was scraped off with a no. 11 surgical scalpel blade held at right angles to the tissue surface. These scrapings were then inoculated onto a medium consisting of Oxoid Blood Agar Base no. 2 with 7% lysed horse blood containing 80 g of polymyxin B per ml (Pfizer Inc.). All plates were incubated for 2 to 3 days at 37°C in anaerobic jars (GasPak, BBL). The procedures described above were at first performed in a flexible plastic anaerobic chamber in an atmosphere of 10% H_2-10% CO_2-80% nitrogen circulated over a palladium catalyst. It was later found that successful isolation of spiral organisms could be achieved without the use of this chamber. Thus, these strictly anaerobic bacteria could survive brief periods of exposure to oxygen. Standard histological techniques were used to demonstrate the presence of organisms that appeared to be morphologically identical, in tissue using electron and phase-contrast microscopy.

Spiral organisms were consistently cultured from the cecal scrapings of all mice and rats examined. For each animal, organisms similar to the dominant morphological types seen in the scrapings were isolated. Experiments with fluorescent antibody are presently in progress to confirm this observation; however, it is proving difficult to raise antibodies against these bacteria.

In the cecal scrapings from a rat, the major organism was a long, thin, spiral organism that appeared to be associated with mucus (Fig. 1a). Thin sections of the cecal mucosa showed what is presumably the same organism colonizing the

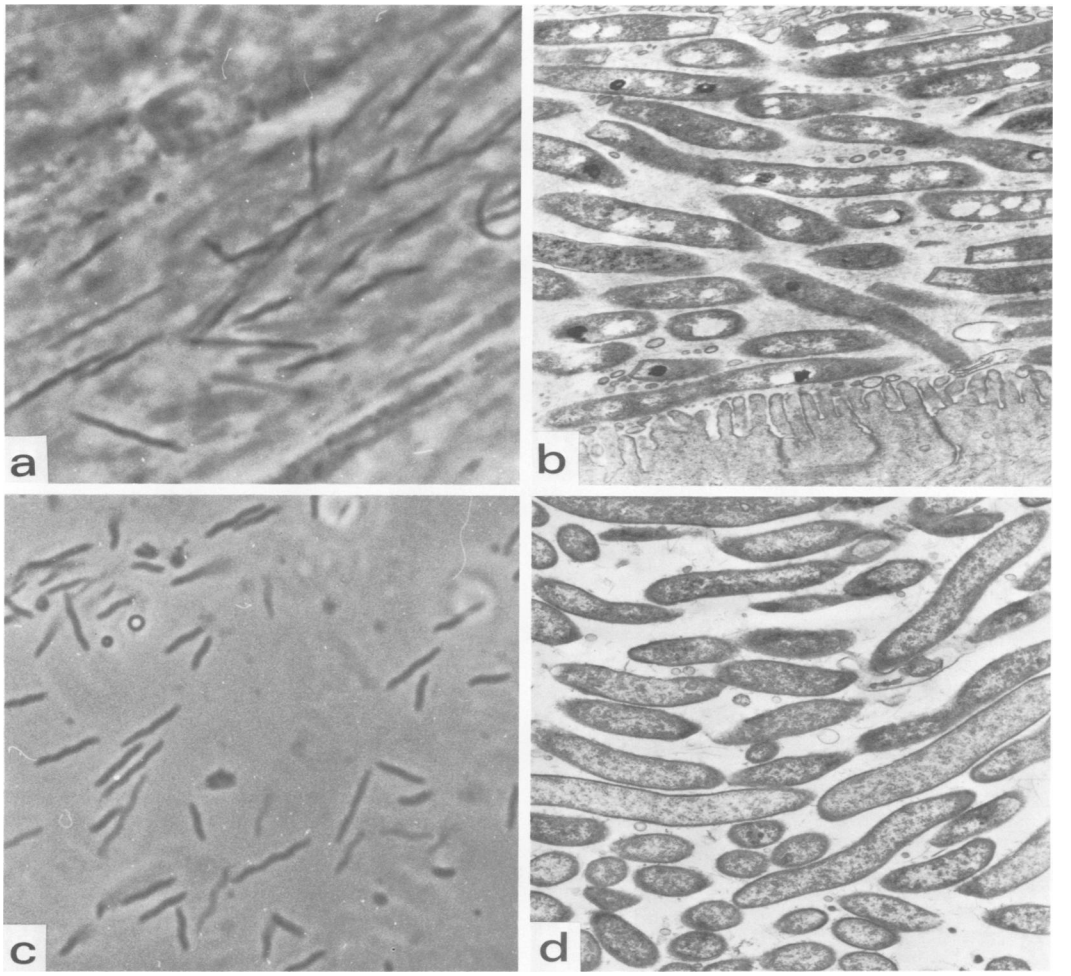

FIG. 1. *Spiral-shaped bacteria associated with the cecal mucosa of rats. (a) Mucosal scrapings (phase contrast, ×2,800); (b) thin section of cecal crypt (electron micrograph, ×20,000); (c) pure culture of isolated bacteria (phase contrast, ×2,800); (d) thin section of cultured bacteria (electron micrograph, ×20,000).*

crypts (Fig. 1b). On culture, this organism grew as small (0.5-mm) discrete colonies with a slight greenish hue. The morphology of the cultured bacterium (Fig. 1c and d) was similar to that seen in the tissues; however, on continued culture, the length became more variable and the spiral became less pronounced. If the plates were wet or the concentration of agar was reduced, the organism spread over the plate making recognition difficult. On plates with no polymyxin, a fine growth of fusiform bacteria completely masked the growth of the spiral.

In the series of mice used for these experiments, the spiral organism colonizing the cecal mucosa had a strikingly different morphology from the organism cultured from rats. The bacterium from the mouse scrapings had two to three spirals of a much greater amplitude than that of the rat (Fig. 2a); this same morphology was also seen in culture (Fig. 2b). The shape of this organism and the presence of classical axial fibrils seen in thin sections indicate that it is a true spirochete (Fig. 2d). In contrast, no axial fibrils were seen in the culture from the rat. Discrete single colonies of the mouse organism were not as commonly seen as with the rat spiral. However, the fine-spreading growth obtained with the mouse organism was present in much greater concentration than other bacteria; therefore, a pure culture was easily obtained. These two cultures have been transferred over 50 times on solid medium in pure culture; however, culture in liquid media has proved difficult. The cultures have also been successfully lyophilized.

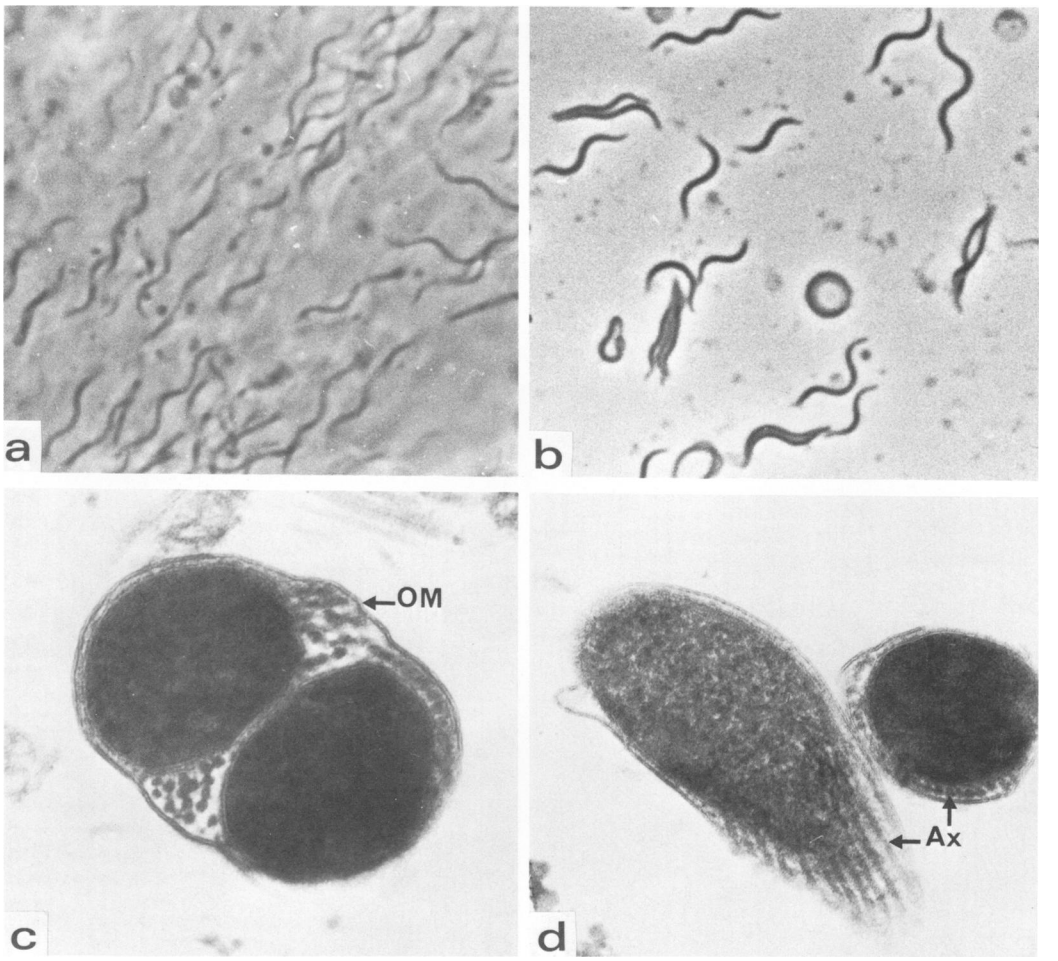

FIG. 2. *Spiral-shaped bacteria associated with the cecal mucosa of mice. (a) Mucosal scrapings (phase contrast, ×2,800); (b) pure culture of isolated bacteria (phase contrast, ×2,800); (c) thin sections of cultured bacteria showing two organisms surrounded by an outer membrane (OM) (electron micrograph, ×120,000); (d) thin sections of cultured bacteria showing axial fibrils (Ax) (electron micrograph, ×95,000).*

Organisms similar to both of these cultured organisms have been described previously in reports of histological studies of intestinal mucosa but have not been cultured (4, 8). Spiral-shaped organisms are sometimes difficult to culture, and elaborate techniques utilizing membrane filters and special growth supplements (1, 2) have been used in the past. The methods described by us are surprisingly simple and, thus, it is worth considering the reasons for our success. The most significant step is the mechanical enrichment achieved by using the well-washed tissue scrapings as the primary inoculum. The spiral bacteria are the dominant organisms in these preparations, thus facilitating their isolation. Without the vigorous saline washing, growth of spirals is difficult to detect and is completely masked by overgrowth of the many other bacterial species that are found in lumen contents. The use of polymyxin was also successful in suppressing the growth of the few fusiform bacteria that remained associated with the washed tissue. Once separated from the competitive effect of other organisms, these spiral bacteria grew well on easily prepared media and, surprisingly, could be handled on the bench rather than manipulated in an anaerobic glovebox.

A number of other spiral-shaped organisms from the intestine also have been grown by us by the above techniques.

The bacteria described here are at present

being further characterized. Certain features are different from those of the spirilla and spirochetes described in the literature. For example, spirochetes from mice had a tendency to be seen in parallel clumps surrounded by an outer membrane. Figure 2c has been included to show this interesting phenomenon that warrants further investigation.

Now that these important mucosal-associated bacteria have been cultured, it is hoped that much can be learned about their contribution to the well-being of the host. These techniques are so successful in culturing a wide range of spiral organisms that they might be applied by other investigators to culture similar organisms that are known to inhabit the intestines of many animals, including humans (5).

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